

SOP 4: Metamorphosis and Monarch Survival

This activity will estimate monarch larva and pupa survival; it should only be done if you have the time and facilities to raise monarchs collected from your monitoring sites. Monarchs often die of causes that are difficult to diagnose, or they may be parasitized by fly or wasp parasitoids that kill them (Monarch Larva Monitoring Project, 2016). Here, we provide information on how to collect data on parasitoids and a protozoan parasite called Oe (*Ophryocystis elektroscirrha*). If you choose to participate in the Oe study, you'll need to contact coordinators at the University of Georgia to obtain a free sampling kit (see instructions below) (Project Monarch Health, 2015).

Collect any 4th or 5th instars each week during your site surveys while conducting SOP 3. You may collect larvae from your monitoring site or other locations (if you collect them from locations other than assigned monitoring sites, keep track of when and where you collected them and note all relevant information on SOP 4 sheet 1). Rear larvae indoors and record whether they survive to adulthood, and, if not, what caused their death (including if cause of death is unknown). If you choose to test butterflies for the Oe parasite, do this before releasing them back at the site. Preserve the adult parasitoids (and the pupal cases) of flies that emerge from the monarchs and mail them to the University of Minnesota for identification.

Equipment and Supplies

- Rearing monarchs:
 - Containers in which to put larvae (see 'Detailed Instruction', step #3 below)
 - Disinfectant (20% bleach-water solution)
 - Lab tape for labeling cages
 - Markers
 - Dental floss
 - Paper towels or filter paper
 - Screen mesh
 - Milkweed to feed caterpillars (collected locally)
 - Cooler for collecting larvae on hot days
- Collecting parasitoid flies and pupariums:
 - Monarch Larva Monitoring Project label
 - Small containers (e.g. pill bottles)
 - Tissues
- Testing for Oe:
 - Oe testing kit requested from Monarch Health (see end of protocol)
 - Disposable gloves
 - Disinfectant (20% bleach-water solution in spray bottle)

Detailed Instructions for Rearing Larvae to Estimate Survival

1. Collecting 4th and 5th instars will minimize the amount of time required to care for them. You may collect and rear eggs or younger larvae, but remember that caring for larvae takes time EVERY DAY (approximately 30-60 minutes), so only collect as many at the appropriate instar stage as you or other staff can care for.
2. If it is over 85°F on a monitoring day, avoid collecting monarch eggs or larvae, or bring a cooler for them. Even if it is under 85°, remember that plastic or glass containers act as small greenhouses, so containers with monarchs should be kept out of the sun, in a cooler, cloth bag, or other opaque carrier. As you collect the monarchs, ensure that you collect the milkweed leaf on which you find them, and enough additional milkweed to last the rest of the day. If the caterpillar is on a stem, gently remove it from the plant and collect a leaf from the same plant you found it on to feed to it.
3. Keep individual larvae in a jar, ice cream bucket, quart-sized deli container, or another container. When they are smaller, they can be in smaller containers (Figure SOP-4.1). The container should be easy to open, and have a screen covering or holes for air flow. It should be large enough for the adult to expand its wings when it emerges. Keep it out of the sun or other hot places (like a car in summer). Keep only one larva in each container to combat disease spread across specimens, and to allow accurate tracking of individual larvae.



Figure SOP-4.1. Example of rearing set-up.
(Photo courtesy of Ilse Gebhard)

4. Clean cages daily. Empty out the caterpillar frass (poop) and old milkweed. Wash your container before using it for another caterpillar using a 20% bleach-water solution.
5. Give larvae fresh milkweed daily. You can pick several days' worth of milkweed, wash it, and keep it in a plastic bag in a refrigerator. It stays fresher if you put a damp piece of paper towel on the bottom of your milkweed bag.
6. 4th and 5th instars that you collect will likely pupate within a week. When they are ready to pupate, they'll crawl to the top of their cage and form a pre-pupal "J" before shedding their skin for the last time (Figure SOP-4.2). You can tell that they will shed their larval skin soon (within minutes) when their tentacles hang very limply and their bodies straighten out a little. Be careful to not jostle the container while larvae are pupating. If the pupae become detached, carefully tie dental floss around the stalk of the pupae (cremaster) and tape it to the top of the cage with masking tape.



Figure SOP-4.2. Pupation sequence. (Photos courtesy of Siah St. Clair)

7. The pupa stage lasts nine to fourteen days. Pupae turn darker the day before butterflies emerge, and look black on the day they emerge (Figure SOP-4.3). At this point, the wings are visible. The butterflies usually emerge in the morning. Their wings will be soft, flexible, and wet when they emerge, but they'll be ready to fly in about 4 hours. If they fall, carefully pick them up by holding the thorax, and hold their legs next to the top or side of the cage. They need to hang with their wings pointed down. A pupa that has been very dark for more than a few days is almost always dead. Within a day of an adult butterfly emerging, release it back at the site from which it was collected after recording information on SOP 4 Data Sheet 1. If you are not able to return to the site, note where it was released instead, recording GPS coordinates (Lat-Long or UTM (NAD 83) and Zone number).

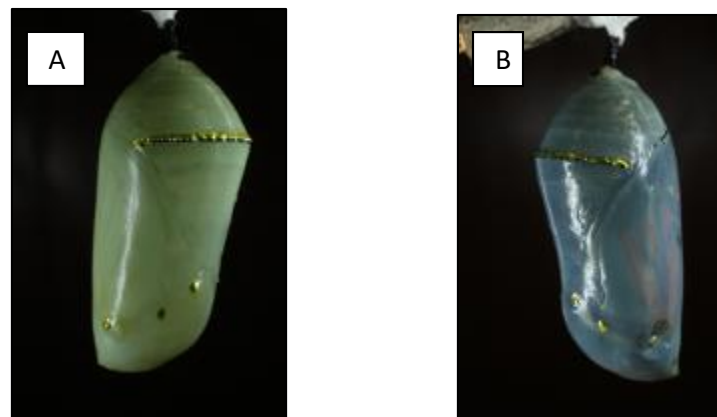


Figure SOP-4.3. Monarch pupae (A) early stage chrysalis/pupae and (B) mature monarch chrysalis/pupae containing monarch close to emergence. Note that wings are visible through the chrysalis wall. (Photos courtesy of Siah St. Clair)

8. Larvae that have been parasitized by flies will often not pupate successfully, but will hang limply and die, although some flies emerge from the pupa. Fly maggots come out of the host larva or pupa on a silk-like thread (Figure SOP-4.4A), and pupate on the bottom of the container. The adult flies emerge about 7-10 days later. Wasps emerge as adults from their host pupa.

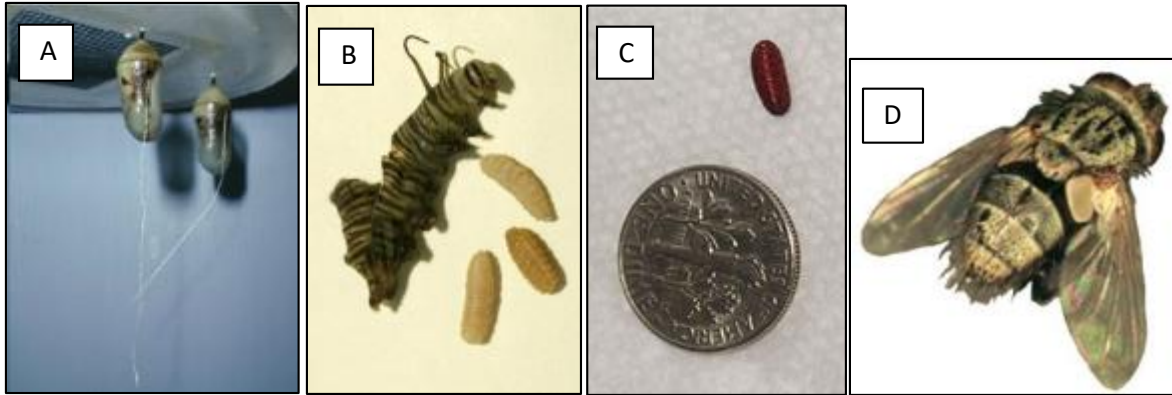


Figure SOP-4.4. Tachinid fly parasitoids of monarchs. (A) Monarch pupae with silk-like thread from tachinid fly parasitoids. (Photo courtesy of Sonia Altizer). (B) Three tachinid fly larvae (maggots) from parasitized caterpillar. (Photo courtesy of Jaap de Roode). (C) Soon after emerging, the flies pupate, turning reddish-brown. (Photo courtesy of Sonia Altizer). (D) Adult tachinid fly (Photo courtesy of UMN Monarch Lab).

9. If larvae die, wait a few days to see if parasitoids emerge from them. Dispose of the monarch larvae carefully (washing your hands after handling them and disposing of them in a different room from where living larvae are kept). Search the dried milkweed leaves for fly pupae.
10. Please send adult parasitoid flies or wasps that come from monarchs you rear to the Monarch Larva Monitoring Project (MLMP). After the **adult flies** emerge from their pupae (7-10 days after they emerge from the monarch), put the flies **and** pupa cases in small containers (e.g. pill bottles, small boxes, Ziploc storage containers), with tissue to prevent them bouncing around. Do not use cotton balls, as the cotton filaments become attached to parts of the fly that are used for identification purposes. If no adult flies emerge, please send us the pupae (with the same information on the label) because we may be able to identify the fly genus or species from the puparium. ***Each container should hold all of the parasitoids that emerged from ONE monarch.*** Put them in a freezer until you have several or until the end of the season. Either label each container with the information noted on the container label below (Figure SOP-4.5), or number the containers and write the information on a separate table sent with the containers. Contact info@monarchlab.org to notify us when a package of flies should be expected. Send the specimens to:

**Monarch Larva Monitoring Project
University of Minnesota
Dept of FWCB
2003 Upper Buford Circle, 135 Skok Hall
St. Paul MN 55108**

Date monarch was collected:
Stage of monarch at collection:
Milkweed species on which monarch was collected:
Location of collection:
of flies and date emerged from monarch*:
Other notes:
**NOT date adult fly emerged from fly pupal case*

Figure SOP-4.5. Container label information.

Instructions for Testing Monarch Adults for Oe (optional)

1. Obtain a sampling kit from [Project Monarch Health](http://ProjectMonarchHealth.org). To request a free kit, email: monarchhealth@gmail.com. You will send the sampling card plus a copy of your data sheet to the University of Georgia (see address below). You will still enter information in SOP 4 sheet 1.
2. Butterflies should not be handled for the first four or five hours after they emerge, and can be kept in the cage until the next day. To sample adult monarchs for the Oe parasite, wear gloves to prevent contamination and change them frequently (after checking every adult monarch). While the parasite is not harmful to humans, it is easily spread from one monarch to another.
3. Remove the butterfly from its rearing container. Hold firmly as shown in the picture below, using a gloved hand (Figure SOP-4.6A). Be sure not to use your other hand to touch the butterfly because that hand will be used to hold the tape sticker and sample for Oe spores. **It is critical that your bare hand NOT touch the butterfly!**
4. Pick up a piece of transparent tape or sticker with your other hand. Gently, but firmly place the sticky side of the piece of tape to the abdomen of the monarch. Press down so that it wraps around and sticks to the sides of the abdomen (Figure SOP-4.6B).
5. Gently peel the tape off and stick it to the index card (Figure SOP-4.6C). You will remove scales in the process, but it will not harm the monarch. Label the tape sample with a number that corresponds to SOP 4 datasheet entry (Figure SOP-4.6D).
6. Sanitize your working surface with 20% bleach-water solution. Thoroughly sterilize container with 20% bleach solution and clean all supplies and tools with bleach before rearing another wild monarch.
7. After you've entered your data, send a copy of the SOP 4 datasheet and the index card to:

**Project Monarch Health
c/o Sonia Altizer
Odum School of Ecology
University of Georgia
Athens, GA 30602**

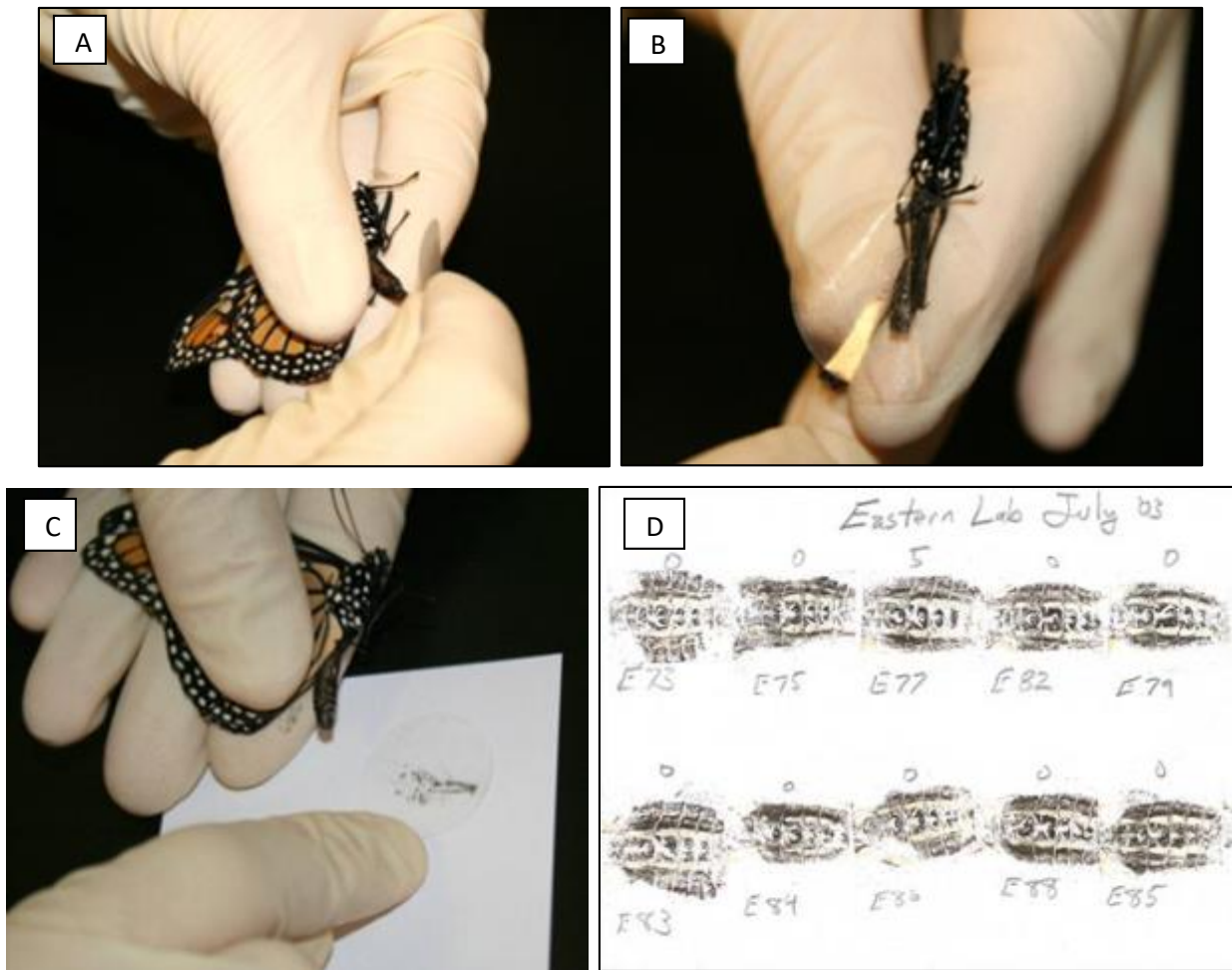


Figure SOP-4.6. Sampling adult monarch for Oe. (A) Hold the monarch gently with gloved hands (B) Wrap tape around the abdomen (C) Gently peel the tape off and stick it to the Oe index card (D) Example of labeled Oe index card.

References

Monarch Larva Monitoring Project. 2016. MLMP. Retrieved May 3, 2016, from <http://www.mlmp.org/>
 Project Monarch Health. 2015. Monarch Health. Retrieved May 12, 2016, from monarchparasites.org